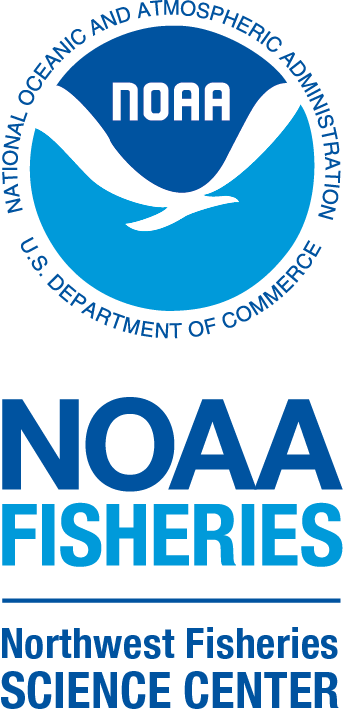
|  |  |
| --- | --- |
| From | Kate’s understanding of the Meeting |
| To | Shallin Busch Paul McElhany, Mike Maher, Danielle Perez |
| Date | Wednesday 2021.03.09 (Last Draft) |
| SUBJ | Respirometry Narrative |
| Drive | https://drive.google.com/drive/folders/1sMiXNW6YCKHZoMop1wlQl-i1HPmBZEEW?usp=sharing |
| REPO |  |
| Study | 2019 Krill Exposure Cross Study |

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Description automatically generatedA picture containing text, clipart

Description automatically generated

**1.0 Respirometry Protocol Description & Refresher**

**1.1.a Respirometry and Project End Goals.** Four lipid samples from each MOAT at the end of the study. Whole body “MORTs” were measured to get a sense of the size for the animals as the study progressed. The termination of the study was biomass dependent.

|  |  |
| --- | --- |
| GitHUB | (Rovinski Repository) |
| https://github.com/KROVINSKI/NWFSC.MUK\_KRL2019respirometry90min | |
|  | |

**1.1.b Project’s End Respirometry Trials** **Determination**

19 vials (volume 28.06mL) were available per round of respirometry

Four trials were conducted over two days

Two blank vials were included per trial

“Krill Doctrine” was employed to determine how best to distribute krill across trials

**Krill Doctrine**

Fast krill for 24hours prior to respirometry and lipids analysis preparation

Choose MOATs with the lowest number of animals remaining for the first round(s) of respirometry and follow on preparation for lipids analysis

Balance between MOATs across days of respirometry

Balance between Treatments when choosing which respirometry round

Must achieve 4 vials per MOATs

Achieve 15-20 animals across treatments for respirometry

Wait until Night Time Conditions begin for the Krill to start Respirometry Trials. The night period for krill began at 1230pm.

**1.1.c Project’s End Respirometry Trials** **Description**

16 Animals per “Ambient Treatment”

16 Animals per “All Change Treatment”

16 Animals per “High Temperature Treatment”

1. Animals per “Current Treatment”

**2.0 Respirometry Project** ““NWFSC.MUK\_KRL2019respirometrySLOPES”

R scripts and documents inside the project listed above was generated to create slopes, intercepts, and apply linear/mixed effects models to the slopes.

**2.1 Merging.** The database is created by merging two datafiles that brought together observer data collected data about individual krill (NIKON SMZ715T with Sony Camera manufactured by Imaging Source (DFK 33UX226c) and IC capture and measure software packages) and the PRESENS (PreSens optical oxygen Fitbox4 sensor). The merged dataframe was referred to as “DRESPmsr”.

|  |  |  |
| --- | --- | --- |
| **2.1a Creating the database** | | |
| Google Drive | dRESP <- read.csv(file = “**KRILL\_Resp\_alltrials.csv**”, stringsAsFactors = FALSE)  dim(dRESP) | dRESP |
| <https://drive.google.com/file/d/19veU1VIWJSrzlMEoVsmDicNNEKG-kWpW/view?usp=sharing> | | |
| Google Drive | dRESPanimal <- read.csv(file = “RespirometryTrials\_all.Animal.Info.csv”)  dim(dRESPanimal) | dRESPanimal |
| <https://drive.google.com/file/d/1vdumqXO0O-3-htxKjtagctW3YLOZoGj3/view?usp=sharing> | | |
| Google Drive | dRESPmsr <- merge(dRESP, dRESPanimal, by=”SensorName”) | dRESPmsr |
| <https://drive.google.com/file/d/1pWiqUsxNBWs5nYindWgORBDG9eMeg4sB/view?usp=sharing> | | |

|  |
| --- |
| **2.1b Merged database dRESPmsr**  **Contains all Animal Specific Information and Probe Measurements** |
| A picture containing text, cup  Description automatically generated |

**2.2 DO Correction.** On Trail 03 of Respirometry Day 02 the PRESENS default temperature of 14°C was entered. All krill under respirometry trials were kept in a water bath between 11.4-12℃. To correct for this temperature correction, Salinity constant was taken from the water supply MOATs 12 (30.3PSU) and modified with the package “wql”. Table below details the units of dissolved of oxygen, including volume inside the incubation vial.

|  |
| --- |
| **2.2a DO Corrections and Oxygen Units**  **Following DO correction “oxygen” units is mg** |
| Diagram  Description automatically generated |
| Vial volumne was .02806. PRESENS probe measured Dissolved Oxygen as mg/L. |

**2.3 Units of Time.** The PRESENS probe initially recording in minutes with decimal time.

|  |
| --- |
| **2.3a Units of Time “delta\_t”**  **Cumulative Amount of Time (.00hr)** |
| Graphical user interface  Description automatically generated |
| Observations were collected from start (T00) to 90minute completion (T90).  The “delta\_t” value will increase until an approximate value of ~1.5hrs.  Simple slopes of krill respiration are recorded as μg / hr.  Slopes will later be corrected for background noise (blank slopes) and krill wet weight. |

**2.4 Animals Per Trial, Per Treatment Tables.** The tables below detail how many animals have been included in each trial 01-04. The tables break down between treatments. The Ambient Treatment was initially included. The Respirometry 90 minute dataframe excludes the Ambient Treatment due a lack of consistent monitoring and equipment logs from both MOATs 04 and MOATs 05. 60 vials in all are included in analysis. With two blanks per trial, 52 animals are comprised those observed across three treatments.

**Trial 1 of 4**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trial1Animals** | **Trial1\_CHG** | **Trial1\_CUR** | | **Trial1\_TMP** | **Trial1\_Blanks** |
| **19** | **5** | **4** | | **8** | **2** |
| All Change  Conditions | Current  Conditions | | | High Temperature  Conditions | |
| 1. “Trial01\_KrilLR16” 2. “Trial01\_KrilLR17” 3. “Trial01\_KrILR110” 4. “Trial01\_KriLR116” 5. “Trial01\_KriLR117” | 1. “Trial01\_KrilLR11” 2. “Trial01\_KrilLR13” 3. “Trial01\_KrilLR18” 4. “Trial01\_KriLR114” | | | 1. “Trial01\_KrilLR14” 2. “Trial01\_KrilLR15” 3. “Trial01\_KriLR111” 4. “Trial01\_KriLR112” 5. “Trial01\_KriLR113” 6. “Trial01\_KriLR115” 7. “Trial01\_KriLR118” 8. “Trial01\_KriLR119” | |
| Two blank vials included to complete a 19 vial respirometry test.  # [1] “Trial01\_KrilLR12”  # [2] “Trial01\_KrilLR19” | | | | | |
| Trail 1 of 4 Loading Start Time | | | 1407 | | |
| Trail 1 of 4 Loading Stop Time | | | 1419 | | |
| Trail 1 of 4 Acclimation Start Time | | | 1419 | | |
| Did figure it was ten minutes from them being loaded | | | | | |
| Trail 1 of 4 Acclimation Stop Time | | | 1424 | | |
| Trail 1 of 4 First Measurement Time | | | "2019-10-28 14:25:03" | | |

**Trial 2 of 4**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trial2Animals** | **Trial2\_CHG** | **Trial2\_CUR** | | **Trial2\_TMP** | **Trial2\_Blanks** |
| **11** | **5** | **4** | | **0** | **2** |
| All Change  Conditions | Current  Conditions | | | High Temperature  Conditions | |
| 1. “Trial02\_KriLR223” 2. “Trial02\_KriLR227” 3. “Trial02\_KriLR229” 4. “Trial02\_KriLR233” 5. “Trial02\_KriLR235” | 1. “Trial02\_KriLR220” 2. “Trial02\_KriLR226” 3. “Trial02\_KriLR232” 4. “Trial02\_KriLR234” | | | character(0) | |
| Two blank vials included to complete a 11 vial respirometry test.  Eight vials were originally collected from the “Ambient Treatment”,  all from MOATs 04 for the second round of respirometry  # [1] “Trial02\_KriLR224”  # [2] “Trial02\_KriLR230” | | | | | |
| Trail 2 of 4 Loading Start Time | | | 1810 | | |
| Trail 2 of 4 Loading Stop Time | | | 1824 | | |
| Trail 2 of 4 Acclimation Start Time | | | 1828 | | |
| Notes about how loading went wrong/awry   Suggest that the minimum number of people working to end the experiment would be four people. Two people stay on lipids and two people stay on respirometry. It became difficult on the second respirometry round to “keep the thread on lipids” and change gears every 15minutes.  Barcodes for the second batch were not printed/generated until the end of the first round. Loading began on the second trial with only one person and without jars already filled with source water. This lead to a longer loading time. | | | | | |
| Trail 2 of 4 Acclimation Stop Time | | | 1849 | | |
| Trail 2 of 4 First Measurement Time | | | "2019-10-28 18:55:23" | | |

**Trail 3 of 4**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trial3Animals** | **Trial3\_CHG** | **Trial3\_CUR** | | **Trial3\_TMP** | **Trial3\_Blanks** |
| **19** | **5** | **4** | | **8** | **2** |
| All Change  Conditions | Current  Conditions | | | High Temperature  Conditions | |
| 1. "Trial03\_KRLr3\_42" 2. "Trial03\_KRLr3\_46" 3. "Trial03\_KRLr3\_48" 4. "Trial03\_KRLr3\_53" 5. "Trial03\_KRLr3\_56" | 1. "Trial03\_KRLr3\_41" 2. "Trial03\_KRLr3\_47" 3. "Trial03\_KRLr3\_51" 4. "Trial03\_KRLr3\_57" | | | 1. "Trial03\_KRLr3\_39" 2. "Trial03\_KRLr3\_40" 3. "Trial03\_KRLr3\_44" 4. "Trial03\_KRLr3\_45" 5. "Trial03\_KRLr3\_49" 6. "Trial03\_KRLr3\_50" 7. "Trial03\_KRLr3\_54" 8. "Trial03\_KRLr3\_55" | |
| Two blank vials included to complete a 19 vial respirometry test.  # [1] "Trial03\_KRLr3\_43"  # [2] "Trial03\_KRLr3\_52" | | | | | |
| Trail 3 of 4 Loading Start Time | | | 1349 | | |
| Trail 3 of 4 Loading Stop Time | | | 1400 | | |
| Trail 3 of 4 Acclimation Start Time | | | 1400 | | |
| Trail 3 of 4 Acclimation Stop Time | | | 1410 | | |
| Trail 3 of 4 First Measurement Time | | | "2019-10-29 14:06:23" | | |

**Trail 4 of 4**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trial4Animals** | **Trial4\_CHG** | **Trial4\_CUR** | | **Trial4\_TMP** | **Trial4\_Blanks** |
| **10** | **1** | **7** | | **0** | **2** |
| All Change  Conditions | Current  Conditions | | | High Temperature  Conditions | |
| 1. "Trial04\_KRLr4\_70" | 1. "Trial04\_KRLr4\_58" 2. "Trial04\_KRLr4\_62" 3. "Trial04\_KRLr4\_65" 4. "Trial04\_KRLr4\_67" 5. "Trial04\_KRLr4\_71" 6. "Trial04\_KRLr4\_75" 7. "Trial04\_KRLr4\_76" | | | character(0) | |
| Two blank vials included to complete a 11 vial respirometry test.  Eight vials were originally collected from the “Ambient Treatment”,  all from MOATs 05 for the second round of respirometry  # [1] "Trial04\_KRLr4\_59"  # [2] "Trial04\_KRLr4\_68" | | | | | |
| Trail 4 of 4 Loading Start Time | | | 1700 | | |
| Trail 4 of 4 Loading Stop Time | | | 1713 | | |
| Trail 4 of 4 Acclimation Start Time | | | 1713 | | |
| Trail 4 of 4 Acclimation Stop Time | | | 1723 | | |
| Did we consider it was 10minutes from the acclimation | | | | | |
| Trail 4 of 4 First Measurement Time | | | "2019-10-29 17:20:23" | | |

**3.0 Timeseries Plots (prior to slope function).** The figures below detail the decrease in oxygen per vial over time in each trial. The figures are broken apart by treatment. The “n/a” treatment refers to blank vials, filled only with filtered seawater from MOATs 12.

|  |
| --- |
| **3.1a Trial 01 Percent Dissolved Oxygen Reduction Timeseries** |
| A picture containing chart  Description automatically generated  A picture containing timeline  Description automatically generated |
|  |

|  |
| --- |
| **3.2 Trial 02 Percent Dissolved Oxygen Reduction Timeseries** |
| Chart, line chart  Description automatically generatedChart, line chart  Description automatically generated |
|  |

|  |
| --- |
| **3.3 Trial 03 Oxygen (μg) Reduction Timeseries** |
| Graphical user interface  Description automatically generated with low confidenceChart  Description automatically generated with medium confidence |
|  |

|  |
| --- |
| **3.4 Trial 04 Oxygen (μg) Reduction Timeseries** |
| Chart, line chart  Description automatically generatedChart, line chart  Description automatically generated |
|  |

|  |
| --- |
| **3.5 All Trials, Across Treatments Oxygen (μg) Reduction Timeseries** |
| Chart  Description automatically generated |
| All trails are included. 52 animals and 8 blank vials are included in the figures above.  [Add notes about different start times  Loading Start Time |Loading Time | Acclimatation Time | Start Time ] |

|  |
| --- |
| **3.6 All Trials, Across Treatments Actual Dissolved Oxygen (mg) Reduction Timeseries** |
| Chart  Description automatically generated |

[update the units for the Y axis (mg/L)]

**4.0 Outlier Removal.** Removing outliers was a six step process to remove observations that had a Cook’s Distance above 4/n. All slopes were included. There was no minimum reduction in oxygen consumption. Positive Slopes were not removed.

The following table details steps to remove outlier observations but not completed slopes:

|  |  |  |
| --- | --- | --- |
| Step | Line Command | Example of the Line Command |
| 1 | Use the unique tool to get names for each krill |  |
| 2 | Create a linear model object for all observations of one krill |  |
| 3 | Using the fortify function, search residuals for cook.sd score for each observation |  |
| 4 | Identify observations with cook.sd score above 4/n |  |
| 5 | Use one dataframe to sort another  (removing the outliers) |  |
| 6 | Rbind back the filtered dataframes |  |
| Number of observations removed 121.  Original dataframe 2007 observations across 52 krill (animals) & eight blank vials  Outliers represent 6.03% of original dataframe’s observations. | | |

5.0 Blank Slope Averages & Correcting Slopes based on blank vials and animal wet weight

|  |  |  |
| --- | --- | --- |
| 1 | Sub setting only the blank vials |  |
| 2 | Creating four different averages based on Trial No. |  |
| 3 | Setting mean values to new variable names |  |
| Blank Mean  Slope Trial 01 | | -4.7994 |
| Blank Mean  Slope Trial 02 | | 0.4331 |
| Blank Mean  Slope Trial 03 | | 0.4478 |
| Blank Mean  Slope Trial 04 | | -.6710 |
| 4 | CorrSlope  Creating  (only subtracting the blank mean values) |  |
| 5 | Correcting for wet animal weight |  |
| “CorrWeightSlope” value factors in both the blank mean (specific to Trial) and the animal wet weight | | |

6.0 Slopes Plots

|  |
| --- |
| 6.1 Slopes Prior to Blank Correction by Treatment (Vial Oxygen (μg)/hr) |
| Chart  Description automatically generated |
| Notice that positive slopes were included. These slopes represent oxygen consumption rates prior to the blank vial background respiration rate correction. |

|  |
| --- |
| 6.2 Slopes with Blank Correction by Treatment (Vial Oxygen (μg)/hr per gram of Krill) |
| Diagram, engineering drawing  Description automatically generated  Units of the Measurement  Positive (amount of oxygen the Krill is consuming)  Take absolute value)  Find paper to reference Krill Units |
| Posit |
| Below is a diagram of the units of measurement (krill slopes):  A picture containing graphical user interface  Description automatically generated |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **7.0 “dSlopes.summary” Table (Corrected Slopes ~ Treatment)** | | | | | | | |
| **Treatment** | **sd** | **mean** | **median** | **IQR** | **n** | **se** | **ci** |
| CHG | 177.1985 | -287.0250 | -252.2802 | 158.8937 | 17 | 42.9769 | 84.2348 |
| CUR | 250.1357 | -383.0114 | -395.8753 | 208.4604 | 19 | 57.3851 | 112.4747 |
| TMP | 180.6342 | -312.2360 | -310.5457 | 279.5354 | 16 | 45.1585 | 88.5107 |

**8.) Corrected Slope Summary Plot**

|  |
| --- |
| **8.1) Corrected Slope Summary Plot**  **((Slope is μg of Vial Oxygen)/hr per Grams of Krill (wet weight in grams))** |
| **Chart, line chart, scatter chart, box and whisker chart  Description automatically generated** |
| There appears to be no significant difference between treatments when corrected against blanks and the individual’s krill wet weight.  [Talk to the uniform conditions that the Krill were ] |
| The purple dots represent the mean- all trials included  Green boxplots show from the 25th percentile to the 75th percentile  error bars +/- SD shown in blue  Confidence Intervals set to 95%  error bars(CI) +/- our confidence intervals (95%) - shown in red |

|  |  |  |
| --- | --- | --- |
| **Avg Corrected Slopes** | **Median Corrected Slopes** | **Corrected Slopes SD** |
| -287.0250 | -252.2802 | 177.1985 |
| -383.0114 | -395.8753 | 250.1357 |
| -312.2360 | -310.5457 | 180.6342 |

**9.0) Mixed Effects Model to Account for Possible MOATs Effect**

|  |
| --- |
| **9.0) Mixed Effects Model to Account for Possible MOATs Effect with the lmer4 package and “lmer” function** |
|  |
| Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']  Formula: CorrWeightSlope ~ Treatment + (1 | MOATS)  Data: dSlopes  REML criterion at convergence: 670.6  Scaled residuals:  Min 1Q Median 3Q Max  -2.5206 -0.5716 0.1458 0.4482 2.4448  Random effects:  Groups Name Variance Std.Dev.  MOATS (Intercept) 0 0.0  Residual 43225 207.9  Number of obs: 52, groups: MOATS, 9  Fixed effects:  Estimate Std. Error df t value Pr(>|t|)  (Intercept) -287.02 50.42 49.00 -5.692 6.95e-07 \*\*\*  TreatmentCUR -95.99 69.41 49.00 -1.383 0.173  TreatmentTMP -25.21 72.42 49.00 -0.348 0.729  ---  Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1  Correlation of Fixed Effects:  (Intr) TrtCUR  TreatmntCUR -0.726  TreatmntTMP -0.696 0.506  optimizer (nloptwrap) convergence code: 0 (OK)  boundary (singular) fit: see ?isSingular |
| This test ordered treatments alphabetically, the All Change Treatment was compared to the Current Conditions and High Temperature Conditions.  No Treatment Effect Found; All Change Treatment compared to Current Conditions (p =0.173) and High Temperature Conditions (p = .0729).  Random Effects Model large variance (43,225) within-treatment level indicates a MOATs effect is relatively small.  **No treatment effect observed, no MOATs effect observed**  **[Look for other models to probe the MOATs question ]** |
| 16 animals from the "All Change" treatment were in included across four trials.  20 animals from the "Current" treatment were in included across four trials.  16 animals from the "High Temperature" treatment were in included across four trials.  (totals only 52 animals) |

|  |
| --- |
| **9.0) Mixed Effects Model to Account for Possible MOATs Effect with the lmer4 package and “lmer” function**  Reordered for Current Conditions Comparison |
|  |
| [1] "CUR" "CHG" "TMP"  Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']  Formula: CorrWeightSlope ~ Treatment + (1 | MOATS)  Data: dSlopes  REML criterion at convergence: 675.8  Scaled residuals:  Min 1Q Median 3Q Max  -2.56088 -0.55055 0.08723 0.55797 1.85699  Random effects:  Groups Name Variance Std.Dev.  MOATS (Intercept) 7820 88.43  Residual 44383 210.67  Number of obs: 52, groups: MOATS, 8  Fixed effects:  Estimate Std. Error df t value Pr(>|t|)  (Intercept) -339.511 69.784 6.067 -4.865 0.00272 \*\*  TreatmentCHG 94.440 96.060 8.323 0.983 0.35325  TreatmentTMP 107.775 107.488 4.947 1.003 0.36251  ---  Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1  Correlation of Fixed Effects:  (Intr) TrtCHG  TreatmntCHG -0.689  TreatmntTMP -0.649 0.447 |
| Direct Comparison of Current Conditions to High Temperature Corrected Slope values (p = .36251) does reveal a significant relationship. |

**[ What will be one table summary ? what all will we include ]**

**Descriptive Stats**

**Statistical review of the fixed effects**